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THE EFFECTS OF A DIET OF POLISHED AND OF UNPOLISHED RICE UPON THE METABOLIC ACTIVITY OF PARAMECIUM.[1]

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The purpose of the following experiments was to discover the effects of the presence and absence of the vitamin contained in rice upon the metabolic activity of *Paramecium*. It was successfully proved by Funk (2), that the vitamin which prevents the deficiency disease known as beri-beri in man is removed from the rice by the process of polishing. Since the lack of this vitamin in the diet of human beings produces a seriously diseased condition it seemed a promising subject for research to determine whether or not the effects could be so far-reaching as to alter the metabolism of a one-celled organism, *Paramecium*. Calkins (1) had previously shown that pancreatic vitamin, a specific in the case of marasmus, had no influence whatever upon the metabolic activities of *Paramecium*. Calkins used the division rate as an index to the vitality of the organism, believing that certain substances in the culture medium may increase the rate of division and others decrease it. This criterion is the one used in the following investigation.

The experiments were performed upon individuals from five pure lines, all from wild cultures started in the laboratory at the same time and kept in spring water and hay infusion for one month. The pure lines were started on December 4, 1917. Each individual was isolated in three drops of distilled water and one drop of 1 per cent. malted milk solution, renewed every other day. The malted milk was prepared by pouring 50 c.c. of boiling distilled water over .5 gram of Horlick's malted milk. After several divisions had taken place each pure line was transferred from the depression slide to a large stock dish containing 200 c.c. of distilled water and 5 c.c. of malted milk solution. Once a week one half of the stock solution was removed and an

[1] From the Biological Laboratory of Bryn Mawr College.

equal amount of fresh water and 5 c.c. of fresh malted milk were added.

In order to eliminate as far as possible the influence of unknown variables every effort was made to prevent contamination of the culture media; graded pipettes were used in measuring the media and the slides containing the individuals were kept in a moist chamber at constant temperature, and removed for observation at approximately the same hour every day. Individuals for the special problem were isolated on March 9, 1918, three being taken from each pure line. Of these three, one was isolated on a depression slide in two drops of distilled water and two drops of 1 per cent. rice water made from polished rice, and a third in two drops of distilled water and two drops of 1 per cent. rice water made from unpolished rice. The rice water was prepared by boiling .5 gram of finely ground rice in distilled water and making up the solution to 1 per cent. after evaporation. The number of divisions was noted at the end of every twenty-four hours, and one individual from each group was isolated on a clean slide in the same medium as before. While the bacterial content is a questionable factor in the experiments, it is controlled to a large extent by the sterilization of slides and pipettes, by the boiling of all media and by the daily isolation of the individuals in fresh media.

Some trial experiments were made for perfection of technique, and then, using eleven days as a fair unit of time, final averages were made of the rates of division in each group. The individuals in malted milk showed the normal rate of division which was slightly higher than that in hay infusion. Peebles (8) has shown that if rightly used a weak solution of malted milk is a most satisfactory culture medium. The metabolic activities of the individuals in polished and in unpolished rice exhibited the influence, not only of the presence and absence of the vitamin, but also of the change of environmental conditions as represented by a new culture medium. Results are given in the accompanying tables.

The number of divisions during the first twenty-four hours of experimentation are not given in the tables, because some time must be allowed for adaptation to new conditions.

TABLE I.

THE DAILY RATE OF DIVISION.

Line.	Medium.	2d Day.	3d Day.	4th Day.	5th Day.	6th Day.	7th Day.	8th Day.	9th Day.	10th Day.	11th Day.
1	Polished rice.....	0	0	1	0	1	0	1	0	0	0
1	Unpolished rice.....	1	0	0	1	1	0	1	0	0	1
1	Malted milk.....	1	1	1	1	1	1	1	1	0	1
2	Polished rice.....	0	1	0	0	1	0	1	0	1	0
2	Unpolished rice.....	0	1	1	1	1	1	1	1	0	1
2	Malted milk.....	1	1	1	1	1	1	1	1	1	1
3	Polished rice.....	0	1	0	1	0	0	1	0	1	0
3	Unpolished rice.....	1	0	0	0	2	0	1	1	0	1
3	Malted milk.....	0	1	0	1	0	2	1	1	1	1
4	Polished rice.....	0	1	0	1	0	0	1	0	0	0
4	Unpolished rice.....	0	1	1	0	1	0	0	1	0	0
4	Malted milk.....	0	1	0	1	1	1	1	1	0	1
5	Polished rice.....	1	0	0	1	0	0	1	0	0	0
5	Unpolished rice.....	1	0	1	1	0	0	1	0	1	1
5	Malted milk.....	1	1	0	1	1	0	0	1	1	2

TABLE II.

TOTAL OF DIVISIONS IN EACH GROUP AND AVERAGES.

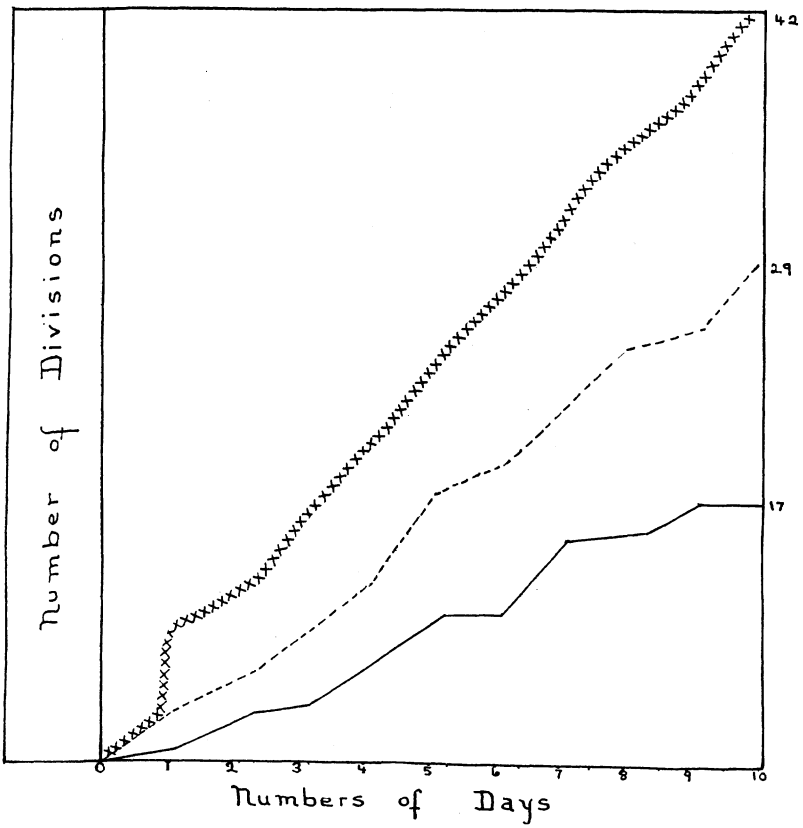
Medium.	Total of Divisions.	10-day Average.	Daily Average.
Polished rice.....	17	3.4	0.34
Unpolished rice.....	29	5.8	0.58
Malted milk.....	42	8.4	0.84

The graph shows that the division rate with polished rice is broken and irregular, while that of the unpolished rice is fairly regular and that of the malted milk very regular in daily increase in the number of divisions. From these results it is evident that unpolished rice is much less favorable than malted milk as a culture medium for *Paramecium*, and that polished rice is quite inadequate for maintaining the vitality of the organisms. It seems probable that the essential factor lacking in the diet of polished rice is the vitamin removed in the process of polishing.

According to McCollum and Davis (4) polished rice is deficient in fat-soluble A, and water-soluble B, the accessory food substances necessary for normal growth and maintenance of life. The fats of rice contain very little if any fat-soluble A, while all the water-soluble B is carried in rice polishings. In using

polished rice as a diet for young rats McCollum and Davis found it necessary to supplement the diet with water-soluble and fat-soluble vitamines. They found that 2 per cent. skim milk powder in combination with the rice supplied nearly enough water-soluble B for normal growth. In the experiments with

TABLE III.



GRAPH SHOWING DAILY TOTAL FOR EACH GROUP.

Crosses indicate divisions of individuals in malted milk. The dotted line indicates divisions of individuals in unpolished rice. The continuous line indicates divisions of individuals in polished rice.

Paramecium the addition of one drop of 1 per cent. malted milk solution to one drop of 1 per cent. polished rice water and two drops of distilled water restored the division rate to the normal

rate shown in malted milk alone. The efficacy of malted milk is due to the presence in it of both water-soluble B and fat soluble A. Unpolished rice contains both but not enough fat-soluble A for maintenance (6).

Since orange juice is so potent a specific in the treatment of deficiency diseases in man, a second set of experiments was undertaken to see if when used with polished rice the accessory food substances in orange juice would be an adequate substitute for the rice vitamin. Parallel experiments were tried with unpolished rice and malted milk to determine whether or not orange juice would increase the metabolic activities of the organism in media already known to be adequate for growth and maintenance.

The orange juice was prepared by boiling 1 c.c. of orange juice in distilled water and making up the solution to 1 per cent. after evaporation. Previous trial experiments had been made with different percentages of the orange juice, 5 per cent., 1 per cent., 2 per cent., and 3 per cent., and malted milk. The results indicated that the 1 per cent. solution would be most favorable for work with *Paramecium*. The races used for the special problem were isolated from various sources, Pure line A was identical with Pure line 1 of the first experiments, Pure line B and Pure line C were isolated from cultures obtained from Powers and Powers, Lincoln, Neb., and the University of Pennsylvania respectively, Pure line D from a wild culture just introduced into the laboratory, and Pure line E from a mixed culture kept in the laboratory in hay infusion all winter. These gave a variety of physiological conditions upon which to experiment. The individuals in Pure lines B and C were much larger and more vigorous than those from the other lines. The wild race was especially small but very vigorous after the firm establishment of the line, while the races which had been kept in the laboratory all winter were small and sluggish. Following the precautions observed in the previous experiments, four individuals from each line were isolated on April 22, 1918, one in two drops of distilled water and one drop of 1 per cent. malted milk solution, a second in one drop of distilled water and two drops of 1 per cent. malted milk solution, a third in one drop of distilled water, one drop of 1 per cent. malted milk solution and one drop of 1 per cent. orange

juice, and a fourth in two drops of distilled water and one drop of 1 per cent. orange juice.

The work was carried on for eleven days, at the end of which time averages were made from the rates of division in each group. The two control groups showed the influence of different degrees of concentration upon the division rate, and the group in orange juice and water was used to determine whether or not orange juice served alone as an adequate food substance. Results are shown in the accompanying tables.

TABLE IV.

THE DAILY RATE OF DIVISION.

(*a*) 2 drops of distilled water and 1 of malted milk.
(*b*) 1 drop of distilled water and 2 of malted milk.
(*c*) 1 drop of distilled water and 1 of malted milk, and 1 of orange juice.
(*d*) 2 drops of distilled water and 1 of orange juice.

	2d Day.	3d Day.	4th Day.	5th Day.	6th Day.	7th Day.	8th Day.	9th Day.	10th Day.	11th Day.
Line A.										
(*a*).....	2	0	1.5	1	2	1	2	2	1	1.5
(*b*)....	2	1	0	1	1	2	2	1	2	0
(*c*).....	2	2	1	2	1	2	2	2	1	1
(*d*).....	1	0	1	1	dead					
Line B.										
(*a*).....	2	1	2	1	1	2	1	0	1	2
(*b*).....	2	1	1	1	2	1	1	1	1	1
(*c*).....	2	1	1	1	1	2	1	0	1	1
(*d*).....	0	0	dead							
Line C.										
(*a*).....	2	1	1	2	1	1	0	1	1	1
(*b*).....	2	1	1	2	1	2	1	1	1	1
(*c*).....	2	1	2	2	1	1	2	1	1	2
(*d*).....	1	0	0	1	0	dead				
Line D.										
(*a*).....	1	1	2	2	2	1	1	1	1	2
(*b*).....	1	0	2	1	2	2	2	2	1	2
(*c*).....	1	2	0	1	3	2	2	2	1	0
(*d*).....	1	1	1	0	dead					
Line E.										
(*a*).....	1	1	1.5	1.5	0	0	2	2	0	2
(*b*).....	2	1	1.5	1.5	0	1	2	1	1	2
(*c*).....	1	1	2	2	0	1	2	1	0	2
(*d*).....	1	1	1	dead						

The small variations in division rate are within the limits of experimental error, with the exception of group (*d*). From these facts it is to be concluded that orange juice is not an adequate food substance, that it does not increase the metabolic

TABLE V.

TOTAL OF DIVISIONS IN EACH GROUP AND AVERAGES.

Line.	*A.*	*B.*	*C.*	*D.*	*E.*	Total.	10-day Average.	Daily Average.
(*a*).....	14	13	11	14	11	63	12.6	1.26
(*b*).....	12	12	13	15	13	65	13	1.3
(*c*).....	16	11	15	14	12	68	13.6	1.36
(*d*).....	3	0	2	3	3	11	2.2	0.22

activities of organisms in malted milk, that the slight variation in the concentration of media employed in these experiments does not alter the rate of division and finally that these conclusions are applicable to races representing a divergence of previous physiological conditions.

TABLE VI.

THE DAILY RATE OF DIVISION.

(*a*) 2 drops of distilled water and 2 of polished rice water.

(*b*) 2 drops of distilled water and 2 of unpolished rice water.

(*c*) 2 drops of distilled water and 2 of polished rice water and 1 of 1 per cent. orange juice.

(*d*) 2 drops of distilled water and 2 of unpolished rice water and 1 of 1 per cent. orange juice.

	2d Day	3d Day.	4th Day.	5th Day.	6th Day.	7th Day.	8th Day.	9th Day.	10th Day.	11th Day.
Line A.										
(*a*).....	0	0	0	1	0	0	0	dead		
(*b*).....	0	1	1	0	0	1	0	0	0	0
(*c*).....	0	0	0	0	0	0	0	dead		
(*d*).....	1	0	0	0	0	0	0	0	0	0
Line B.										
(*a*).....	0	0	0	0	1	0	0	dead		
(*b*).....	0	2	0	0	1	0	0	0	0	dead
(*c*).....	1	0	0	1	0	0	0	0	0	dead
(*d*).....	1	0	0	1	0	0	0	0	0	0
Line C.										
(*a*).....	0	0	0	0	0	dead				
(*b*).....	0	0	1	0	0	0	dead			
(*c*).....	0	0	0	0	0	0	0	0	dead	
(*d*).....	0	1	0	0	0	0	1	0	dead	
Line D.										
(*a*).....	0	0	0	0	0	dead				
(*b*).....	0	0	1	0	0	dead				
(*c*).....	0	0	0	0	0	0	0	0	0	0
(*d*).....	0	0	0	0	1	0	0	0	0	0
Line E.										
(*a*).....	1	0	0	0	dead					
(*b*).....	0	1	0	0	1	dead				
(*c*).....	1	0	0	1	dead					
(*d*).....	1	0	0	0	1	0	1	0	0	1

TABLE VII.

TOTAL OF DIVISIONS IN EACH GROUP AND AVERAGES.

Line.	*A.*	*B.*	*C.*	*D.*	*E.*	Total.	10-Day Average.	Daily Average.
(*a*).....	1	1	0	0	1	3	0.6	0.06
(*b*).....	3	3	1	1	2	10	2.0	0.2
(*c*).....	0	2	1	1	2	6	1.2	0.12
(*d*).....	1	2	2	1	4	10	2.0	0.2

The experiments with rice and orange juice were begun May 6, by isolating four individuals from Lines A, B, C and D, two as controls in two drops of distilled water and two drops of 1 per cent. rice water, from polished and unpolished rice respectively, and two like the controls with the addition of one drop of 1 per cent. orange juice to each. The work was carried on for eleven days at the end of which time averages were made of the rates of division in each group, to determine whether or not the substitution of the accessory food substances in orange juice for the rice vitamin had increased the metabolic activities of the individuals in polished rice, and also to note any stimulus to division produced by the addition of orange juice to unpolished rice. The results are shown in the accompanying tables.

It is evident from these results that all of the *Paramecia* were in a state of depression which was not altered to any appreciable extent by the presence of orange juice, although the individuals in unpolished rice and orange juice seem to have had more resistance to the unknown factors which produced dissolution in the other groups. The orange juice had a slightly stimulating effect upon the metabolic activities of the organisms in polished rice but absolutely no effect whatever upon the division rate of those in unpolished rice. From these results it may be concluded that orange juice has not a powerful influence upon the metabolic activities of *Paramecium*.

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